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IL-6 Promotes Cardiac Graft Rejection Mediated by CD4+ Cells

Adam Jared Booth,*1 Svetlana Grabauskiene,†1 Sherri Chan Wood,†1 Guanyi Lu,‡ Bryna E. Burrell,‡ and D. Keith Bishop‡

IL-6 mediates numerous immunologic effects relevant to transplant rejection; however, its specific contributions to these processes are not fully understood. To this end, we neutralized IL-6 in settings of acute cardiac allograft rejection associated with either CD8+ or CD4+ cell-dominant responses. In a setting of CD8+ cell-dominant graft rejection, IL-6 neutralization delayed the onset of acute rejection while decreasing graft infiltrate and inverting anti-graft Th1/Th2 priming dominance in recipients. IL-6 neutralization markedly prolonged graft survival in the setting of CD4+ cell-mediated acute rejection and was associated with decreased graft infiltrate, altered Th1 responses, and reduced serum alloantibody. Furthermore, in CD4+ cell-dominant rejection, IL-6 neutralization was effective when anti–IL-6 administration was delayed by as many as 6 d posttransplant. Finally, IL-6-deficient graft recipients were protected from CD4+ cell-dominant responses, suggesting that IL-6 production by graft recipients, rather than grafts, is necessary for this type of rejection. Collectively, these observations define IL-6 as a critical promoter of graft infiltration and a shaper of T cell lineage development in cardiac graft rejection. In light of these findings, the utility of therapeutics targeting IL-6 should be considered for preventing cardiac allograft rejection.


Immune responses directed against transplanted tissues are the major obstacle to graft acceptance and survival. Numerous cell types can contribute to alloimmune responses that culminate in cardiac graft rejection, although CD4+ T cells in particular play a central role (1–4). T cell responses are influenced by context, including the local cytokine milieu. Recent findings have broadened our understanding of how the pleiotropic cytokine IL-6 can affect T cell responses, including the inhibition of Th1 responses and enhancement of Th2 and Th17 lineage development (5–9). Other reports indicate that IL-6 promotes or augments established Th1 responses (10, 11). Furthermore, IL-6 may disrupt mechanisms of immune tolerance by inhibiting suppressive functions of regulatory T cells (12–14). Hence, the role of IL-6 in Th subset balance remains controversial and its role in shaping immune responses is complex and likely context-dependent.

Increased intragraft IL-6 expression has been associated with histologic rejection in patients (15, 16) and in experimental models of acute (17) and chronic (18, 19) allograft rejection, although its specific contributions to the evolution of rejection responses are not clearly defined. IL-6 has been reported to function as a graft-produced “danger signal” that promotes acute graft rejection (17).

IL-6 may also act in concert with IL-17 to override established graft tolerance in response to TLR activation (20). Indeed, neutralizing IL-6 prevents development of Th17 responses responsible for costimulation blockade-resistant rejection in Tbet−/− allograft recipients (21, 22). Additionally, we have recently described a critical role for IL-6 in chronic allograft rejection mediated by CD4+ cells (18, 19, 23).

CD4+ cell help is required for the development of CD8+ Th1-dominated responses associated with unmodified acute rejection (1). In contrast, when CD8+ cells are eliminated by in vivo depletion, the remaining CD4+ cells mount a Th2-dominated rejection response that is characterized by eosinophilic infiltrate (24–26). Because IL-6 has been reported to mediate Th2 responses (6, 7), but may also promote Th1 responses (10, 11), we sought to clarify the effects of IL-6 in the development of transplant rejection in models where alloimmune responses are dominated by CD8+ Th1 responses or CD4+ Th2 responses.

Materials and Methods

Culture media

Cells were cultured in RPMI 1640 supplemented with 2% FCS, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, 1.6 mM l-glutamine, 10 mM HEPES buffer (Invitrogen, Carlsbad, CA), 0.27 mM l-asparagine, 1.4 mM l-arginine HCl, 14 μM folic acid, and 50 μM 2-ME (Sigma-Aldrich, St. Louis, MO).

Mice

Female wild-type (WT) C57BL/6 (H-2b) and BALB/c (H-2d) mice were purchased from Charles River Laboratories (Raleigh, NC). IL-6−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All animals were kept under microisolator conditions as reviewed and approved by the University of Michigan’s Committee on Use and Care of Animals.

Vascularized cardiac transplantation

Heterotopic cardiac transplantation was performed as described (27). Briefly, the aorta and pulmonary artery of a BALB/c donor heart were anastomosed end-to-side to a C57BL/6 recipient’s abdominal aorta and inferior vena cava, respectively. Upon perfusion with the recipient’s blood, the transplanted heart resumes contraction. Graft function was monitored by abdominal palpation.
In vivo mAb therapy

Anti-CD8 (hybridoma 2.43) and anti–IL-6 (hybridoma MP5-20F3; with permission of DNAX) were obtained from the American Type Culture Collection (Manassas, VA). mAbs were purified and resuspended in PBS by Bio X Cell (West Lebanon, NH). Control rat IgG Ab was purchased from Sigma-Aldrich. Where indicated, cardiac allograft recipients received 1 mg anti-CD8 mAb i.p. on days −1, 0, and 7 to deplete CD8+ cells. Anti–IL-6 mAb or control rat IgG were administered by i.p. injection of 1 mg on days −1, 1, and 3 except for delayed administration experiments with anti–IL-6, where the same dosing regimen commenced on day 2, 4, or 6 posttransplant.

Histologic analysis

Formalin-fixed and paraffin-embedded grafts were sectioned and stained with H&E to assess myocardial viability (presence of cross-striation and myocyte nuclei) and the nature, intensity, and localization of graft-infiltrating cells (GIC).

GIC harvest and analysis

 Portions of three transplanted hearts were removed, pooled, minced, and digested with 1 mg/ml collagenase A (Roche Diagnostics, Penzberg, Germany) for 30 min at 37˚C. After tissue debris settled, suspended GIC were harvested. RBC were lysed by hypotonic shock and GIC were passed through a 30-μm nylon mesh. Viable cells were counted by trypan blue exclusion. Cytocentrifuged GIC were Wright stained for differential enumeration.

ELISPOT assay

ELISPOT assays were performed as previously described (28). Capture and detection mAb pairs (BD Biosciences, San Jose, CA) were as follows: IFN-γ (R4-6A2, XMG1.2), IL-4 (11B1, BV6D-24G2), and IL-17 (TC11-18H10, TC11-8H4.1). Polyvinylidene difluoride-backed microtiter plates (Millipore, Billerica, MA) were coated with unlabeled mAb and blocked with 1% BSA in PBS. Irradiated (1000 rad) BALB/c splenocytes (4×10^5) and 1×10^6 recipient splenocytes were added to each well for 24 h. After washing, biotinylated detection mAbs were added to the plates. Plates were washed and a 1:1000 dilution of anti-biotin alkaline phosphatase conjugate (Vector Laboratories, Burlingame, CA) was added to IFN-γ and IL-17 plates, and a 1:2000 dilution of HRP-conjugated streptavidin (Dako, Carpinteria, CA) was added to IL-4 plates. Plates were washed and spots were visualized by addition of NBT (Bio-Rad Laboratories, Hercules, CA)/3-bromo-4-chloro-indolyl phosphate (Sigma-Aldrich) to IFN-γ and IL-17 plates, or 3-amin-9-ethylcarbazole ( Pierce) to IL-4 plates. Color development continued until spots were visible and stopped by adding H2O. Plates were dried and spots were quantified with an ImmunoSpot series 1 ELISPOT analyzer (Cellular Technology, Shaker Heights, OH).

Quantitative real-time PCR

Graft RNA was isolated by homogenizing tissues in TRIzol reagent (Invitrogen) as per the manufacturer’s protocol. Five micrograms of total RNA was reverse transcribed using oligo(dT), 2′-deoxyxycytidine 5′-triphosphate, Moloney murine leukemia virus reverse transcriptase (Invitrogen), and RNAsin (Promega, Madison, WI) in PCR buffer (Roche, Indianapolis, IN). The cDNA was purified by a 1:1 extraction with phenol/chloroform/isomyl (25:24:1) (Invitrogen) and precipitated in 0.1 vol 3 M NaOAc and 2 vol absolute ethanol. MCP-1 transcripts were quantified by quantitative real-time PCR using SYBR Master Mix (Bio-Rad Laboratories) in a Rotor-Gene 3000 thermocycler (Corbett Life Science, San Francisco, CA). Relative expression levels were normalized to GAPDH expression using the Rotor-Gene comparative concentration utility.

Primers sequences were as follows: GAPDH (Gapdh) forward, 5′-CTGG-TGTGATATGTCTGGT-3′, reverse, 5′-CAGTCTTCTGAGTCACCTG-3′; MCP-1 (Ccl2) forward, 5′-TTAACGCCACACCTCAGCTG-3′, reverse, 5′-GCCTTCTGGGACACCTGCTG-3′.

Quantification of graft reactive alloantibodies

Graft-reactive alloantibodies were quantified as previously described (24, 29). Briefly, 1×10^6 P815 (H-2 d) cells (American Type Culture Collection) were incubated with a 1:50 dilution of sera followed by FITC-conjugated rabbit anti-mouse IgG Ab (Zymed, San Francisco, CA). Unfixed samples were analyzed by flow cytometry. Data are reported as the mean channel fluorescence.

Statistical analysis

Allograft survival curves were analyzed using a log-rank test. Significance of ELISPOT and alloantibody results was determined by an unpaired test with Welch’s correction. All data were analyzed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). A p value of ≤0.05 was considered statistically different.

FIGURE 1. Neutralizing IL-6 delays CD8+ cell-dominated allograft rejection and inverts the Th1/Th2 balance in CD4+ and CD8+ replete recipients. C57BL/6 recipients of BALB/c cardiac grafts were treated with neutralizing anti–IL-6 mAb or control rat IgG perioperatively and were euthanized at the survival of six cardiac allografts. (A) IHC and (B) IF data showing in vivo neutralization of IL-6 in recipients treated with anti–IL-6 mAb (3) or control rat IgG Abs ( ). Each curve represents the survival of six cardiac allografts. B. Mean GIC number from cardiac allografts harvested at the time of rejection whose recipients received rat IgG or anti–IL-6. Bars represent mean ± SEM. C. GIC composition expressed as percentage of lymphocytes, macrophages, eosinophils, and neutrophils. Bars represent the mean percentage of five counts of 100 cells from Wright-stained cytospins representing GIC pooled from at least six individual grafts. *p = 0.0142. D, Donor-reactive cytokine responses at time of allograft harvest by splenocytes harvested from allograft recipients treated with neutralizing anti–IL-6 mAb or control rat IgG Abs as quantified by ELISPOT. Individual points represent the mean of triplicate cultures performed with splenocytes from individual allograft recipients. Lines represent the mean of points shown, and naive (untransplanted) controls are shown for reference.
Results

IL-6 neutralization delays CD8⁺ cell-dominant acute graft rejection and alters alloimmune responses

In mice replete with both CD4⁺ and CD8⁺ cells, rejection is dominated by CD8⁺ cells, which mount a Th1 response (24). Allografts in recipients treated with neutralizing anti–IL-6 mAb survived significantly longer than did those treated with control rat IgG (Fig. 1A). The increased survival time of grafts in recipients receiving anti–IL-6 mAb was associated with a significant reduction in the number of GIC (Fig. 1B). Further evaluation of GIC revealed a significant increase in the percentage of eosinophils in grafts whose recipients received IL-6 neutralizing therapy (Fig. 1C), suggesting increased Th2 responses (24). We further observed an increase in graft-reactive Th2 responses by ELISPOT assay, evidenced by increased IL-4 production by recipient splenocytes (Fig. 1D). Increased Th2 responses associated with anti–IL-6 mAb treatment were also correlated with decreased donor-reactive Th1 responses (Fig. 1D), similar to a previous report linking IL-6–deficient grafts to decreased IFN-γ responses when recipient splenocytes were stimulated with anti-CD3 mAb (17). Although IL-6 has been linked with Th17 responses (8, 9, 20, 22), splenocyte production of IL-17 was negligible in both groups (Fig. 1D), suggesting that IL-6 contributes to acute allograft rejection through Th17-independent pathways.

Allograft rejection dominated by CD4⁺ cell responses requires IL-6

It is well established that CD4⁺ cells mediate acute rejection independent of CD8⁺ cells (24, 30). The heightened Th2 responses in recipients receiving IL-6 neutralizing therapy (Fig. 1D) were reminiscent of previous observations with allograft recipients depleted of CD8⁺ cells or lacking IFN-γ, both of which reject grafts with associated Th2 immune responses (24, 31). We therefore asked whether IL-6 was necessary for acute rejection of cardiac grafts mediated by CD4⁺ T cells. To this end, we treated allograft recipients that were depleted of CD8⁺ cells with anti–IL-6 or control rat IgG. Inductive treatment with anti–IL-6 mAb prolonged survival of most grafts for at least 50 d posttransplant (Fig. 2A) in recipients depleted of CD8⁺ cells. Long-term graft survival was associated with a significant decrease in GIC number (Fig. 2B, 2C).

We next asked whether decreased graft infiltration in recipients receiving anti–IL-6 was associated with changes in Th1, Th2, or Th17 priming. Whereas Th2 and Th17 priming remained unchanged, IL-6 neutralization caused a small but significant decrease in Th1 responses compared with rat IgG-treated controls (Fig. 2D). The striking difference in GIC number compared with the more modest reduction in Th1 cytokine production led us to consider that the primary effect of anti–IL-6 delaying CD4⁺-mediated graft rejection might be preventing graft infiltration rather than preventing T cell priming.

Posttransplant administration of anti–IL-6 mAb prevents CD4⁺ cell-mediated graft rejection

IL-6 neutralization markedly inhibited graft infiltration in both CD8⁺ and CD4⁺ cell-dominated rejection (Figs. 1B, 2C). In the setting of CD4⁺ cell-mediated rejection, anti–IL-6 not only reduced graft infiltration but also promoted long-term graft sur-

![FIGURE 2.](http://www.jimmunol.org/)

Neutralizing IL-6 abrogates CD4⁺ cell-mediated rejection. C57BL/6 recipients of BALB/c cardiac grafts were depleted of CD8⁺ cells and received perioperative anti–IL-6 mAb or control rat IgG. A, Allograft survival in CD8⁺ cell-depleted recipients receiving anti–IL-6 (○) or control rat IgG (●). B, Representative views of H&E-stained sections from allografts in A. Note the intense perivascular infiltrate and parenchymal cell death in grafts from recipients treated with control rat IgG, whereas grafts from recipients receiving anti–IL-6 mAb exhibit markedly less infiltrate and parenchymal cell death. Original magnification ×400. C, Mean GIC number from rat IgG or anti–IL-6–treated recipients. D, Donor-reactive cytokine responses quantified by ELISPOT assays performed on splenocytes harvested from graft recipients at time of allograft rejection (rat IgG control group) or day 50 for allografts surviving long-term (anti–IL-6–treated group). Points represent the mean of triplicate cultures performed with splenocytes from individual transplant recipients. Lines represent the mean of points, and naive (untransplanted) controls are shown for reference.
vival (Fig. 2A). Therefore, we hypothesized that the essential contribution of IL-6 in this setting might be promoting lymphocyte homing to the graft rather than affecting alloimmune response priming. To better understand these roles of IL-6, we delayed the administration of anti–IL-6 by as many as 6 d to assess the function of IL-6 after early events of TH priming (1).

When anti–IL-6 was administered 2 d posttransplant, grafts survived for at least 50 d in recipients depleted of CD8+ cells (Fig. 3A). Interestingly, delay of anti–IL-6 administration to 4–6 d posttransplant resulted in most grafts surviving for 50 d (Fig. 3A). Hence, delayed neutralization of IL-6 also markedly prolonged graft survival. As with perioperative administration, delayed administration of anti–IL-6 significantly reduced infiltrating lymphocytes in grafts harvested at day 50 posttransplant (Fig. 3B), demonstrating that delayed IL-6 neutralization effectively inhibited graft infiltration. Delayed IL-6 neutralization also resulted in reduced TH1 responses at day 50 posttransplant (Fig. 3C), similar to perioperative neutralization of IL-6 in CD8- cell-depleted recipients.

**Early effects of IL-6 neutralization in preventing CD4+ cell-dominant rejection**

In the context of CD4+ cell-dominant graft rejection, IL-6 neutralization significantly prolonged graft survival, with most grafts surviving for at least 50 d. In contrast, control rat IgG-treated recipients rejected their grafts by day 14 (Figs. 2A, 3A). Because of the broad difference in survival times of these groups, we sought a more direct comparison by evaluating both groups at day 14 posttransplant. We further compared these time points to recipients harvested at day 6 posttransplant to allow initial T cell priming to occur and to better assess the influence of anti–IL-6 therapy between day 6 and day 14 (Fig. 4). Graft-reactive TH1 responses at day 14 posttransplant were decreased in recipients receiving delayed anti–IL-6 mAb (beginning on day 6 posttransplant) in comparison with both day 14 rat IgG-treated controls and CD8-depleted recipients harvested at day 6 posttransplant (Fig. 4A). Consistent with enumeration of GIC at later time points (Fig. 3B), anti–IL-6 treatment reduced graft infiltration by day 14 posttransplant in comparison with both day 14 rat IgG controls and CD8-depleted recipients harvested at day 6 posttransplant (Fig. 4B). This suggests that IL-6 neutralization was able to decrease the number of GIC between day 6 and day 14 posttransplant. Taken together, these observations suggest that delayed IL-6 neutralization recapitulates the effects of perioperative neutralization.

**IL-6 neutralization decreases intragraft expression of MCP-1 and production of graft-reactive alloantibody in CD4+ cell-dominant rejection**

Because IL-6 neutralization was associated with reduced graft infiltration (Figs. 1B, 2C, 3B, 4B), we asked whether IL-6 neutralization might be affecting MCP-1 expression, as IL-6 has been previously described to promote MCP-1 production (32–34). Indeed, intragraft expression of MCP-1 was significantly reduced in grafts of recipients receiving delayed anti–IL-6 mAb compared with controls (Fig. 4C). In addition to its ability to modulate chemokine expression, IL-6 has long been known to augment Ab responses (35). Allograft rejection in recipients depleted of CD8+ cells is associated with Th2 responses (24). Although Th2-associated eosinophils may directly participate in transplant rejection (24, 36, 37), Th2 responses are also associated with Abs that may play a role in cardiac rejection (38–40). We therefore considered that one effect of IL-6 neutralization in CD4+ cell-dominant rejection may be decreased graft-reactive Ab responses. Quantification of graft-reactive alloantibody in recipient sera revealed that delayed IL-6 neutralization was associated with decreased levels of circulating graft-reactive IgG when compared with recipients treated with control Abs (Fig. 4D). Hence, IL-6 may further contribute to graft rejection by augmenting the production of anti-donor Ab.

**Graft-protective effects of IL-6 neutralization are recapitulated in IL-6- deficient recipients**

IL-6 neutralization is expected to affect both graft and recipient sources of IL-6 in allograft recipients depleted of CD8+ cells. Hence, we asked whether IL-6- / recipients would reject their grafts similarly to WT recipients in CD4+ cell-dominant rejection. In this setting, grafts in IL-6- / recipients survived comparably to grafts in recipients receiving anti–IL-6 mAb, with most grafts surviving >50 d (Fig. 5A).

**FIGURE 3.** Delayed administration of anti–IL-6 mAb abrogates CD4+ cell-dominant rejection. C57BL/6 recipients of BALB/c cardiac grafts were depleted of CD8+ cells and treated with neutralizing anti–IL-6 mAb administered perioperatively or beginning on day 2, 4, or 6 posttransplant and compared with those receiving control rat IgG. Experimental samples were harvested at the time of allograft rejection (rat IgG control group) or day 50 for allografts surviving long-term (delayed anti–IL-6-treated groups). A, Allograft survival in recipients depleted of CD8+ cells and given anti–IL-6 mAb either perioperatively (squares) or commencing on day 2 posttransplant (cross bars), day 4 posttransplant (triangles), or day 6 posttransplant (diamonds) compared with control rat IgG (circles). B, Mean GIC number from groups described in A. *p = 0.0228 for day 2 anti–IL-6 versus rat IgG; *p = 0.0240 for day 6 anti–IL-6 versus rat IgG. Bars represent the mean ± SEM. C, ELISPOT quantification of donor-reactive cytokine production by recipient splenocytes at time of graft harvest. Points represent the mean of triplicate cultures performed with splenocytes from individual mice. Lines represent the mean of points. *p = 0.0060 for day 2 anti–IL-6 versus rat IgG; *p = 0.0063 for day 6 anti–IL-6 versus rat IgG.
in WT mice, increased graft survival in IL-6 \(^{-/-}\) allograft recipients was associated with significantly reduced GIC numbers (Fig. 5B) and Th1 responses (Fig. 5C). Further, whereas WT recipients receiving IL-6 neutralization exhibited unchanged Th2 responses associated with IL-6 neutralization in WT recipients (Fig. 2D), Th2 responses in IL-6 \(^{-/-}\) recipients were decreased (Fig. 5C). In summary, these results indicate that recipient IL-6 production is of critical importance for graft infiltration and the shaping of T helper responses associated with CD4\(^{+}\) cell-dominant graft rejection.

**Discussion**

IL-6 has been implicated in acute and chronic cardiac rejection (17–19), as well as the disruption of established allograft tolerance (20). Broader immunologic impacts of IL-6 may also affect transplant outcome, including its effects on T cell survival (41), chemotaxis of CD4\(^{+}\) cells (42), priming of CD8\(^{+}\) cell responses (43–45), and direction of T cell responses (5, 7–9, 13, 14). To better understand the role of IL-6 in the evolution of T cell responses against cardiac grafts, we investigated the therapeutic potential of IL-6 neutralization in models of acute rejection dominated by either CD8\(^{+}\) or CD4\(^{+}\) cell responses.

IL-6 neutralization prolongs allograft survival in otherwise unmodified rejection (17), which is dominated by CD8\(^{+}\) Th1 responses (24, 26). Our investigation of CD8\(^{+}\) cell-dominant acute rejection indicates that prolonged allograft survival associated with IL-6 neutralization coincided with decreased GIC number. Furthermore, anti–IL-6 decreased IFN-\(\gamma\) (Th1) responses and concomitantly increased IL-4 (Th2) production by recipient splenocytes. This reversal of the Th1/Th2 response balance was consistent with increased eosinophilic GIC content, a characteristic of Th2-mediated graft rejection (24, 37). Thus, although IL-6 is not required for unmodified allograft rejection by CD8\(^{+}\) cells, IL-6–mediated effects are integral in shaping anti-graft Th responses and graft infiltration.

To further investigate the role of IL-6 in shaping the Th1/Th2 balance in immune responses against cardiac allografts, we neutralized IL-6 in an acute rejection setting characterized by Th2-mediated rejection dominated by CD4\(^{+}\) cells (24). In this context, IL-6 neutralization promoted long-term allograft acceptance.
MCP-1 is a chemoattractant for multiple cell types that is upregulated by IL-6 (32, 33) and expressed persistently in cardiac allografts (49). The importance of MCP-1 in allograft rejection has been further demonstrated, as cardiac allograft survival can be prolonged by viral transduction of a vector encoding a MCP-1 inhibitor (50).

In addition to directing lymphocyte homing, IL-6 is also a potent modulator of B cell biology (35). Indeed, acute Ab-mediated rejection is a significant obstacle for the survival of human allograft recipients (38, 51). Graft-reactive IgG in recipient serum was significantly decreased in response to IL-6 neutralization. Recent in vitro findings suggest that IL-6 induces Ab production indirectly by promoting the production of IL-21 by CD4+ cells (52). Note that the ability of IL-6 to promote IL-21 in CD4+ cells may have implications beyond providing help for Ab production (53), as IL-21 also promotes CD4+ cell differentiation, CD8+ cell proliferation and activity, and NK cell cytotoxicity (54). Our findings solidify the pleiotropic identity of IL-6, as neutralization prolongs graft survival by opposing Th1 priming, graft infiltration, and humoral responses against the graft. These effects likely synergize to promote graft survival.

A previous investigation has implicated graft production of IL-6 in unmodified acute cardiac rejection (17), which is dominated by CD8+ cells (24, 26). However, the multifaceted effects of IL-6 on CD4+ cell-dominant graft rejection include processes that likely occur in the periphery of allograft recipients. This led us to assess the responses of IL-6−/− recipients in CD4+ cell-dominant graft rejection. Effects of IL-6 neutralization were recapitulated in IL-6−/− recipients, indicating that recipient origin IL-6 is required for CD4+ cell-mediated graft rejection. Curiously, splenocytes from IL-6−/− graft recipients exhibited reduced Th1 and Th2 responses whereas splenocytes from recipients treated with anti–IL-6 had decreases in Th1 alone. A possible explanation for these differences could be that cells removed from recipients receiving anti–IL-6 are able to produce IL-6 in ELISPOT culture, whereas IL-6−/− splenocytes cannot. This would further support a context-dependent role for IL-6 in both Th1 and Th2 immune responses. Nonetheless, these data are consistent with the requirement for recipient, rather than donor-derived, IL-6 in CD4+ cell-mediated allotransplantation. Whereas recipient production of IL-6 reportedly does not promote unmodified graft rejection (17) dominated by CD8+ cells (24, 26), recipient origin IL-6 is necessary for CD4+ cell-dominant graft rejection, suggesting that CD4+ and CD8+ cells have differential requirements for IL-6 in generating responses to cardiac allografts.

Although IL-6 neutralization prolonged graft survival with different efficacies in models of CD4+ and CD8+ cell-dominant acute rejection, several effects of anti–IL-6 were consistent in both models. First, IL-6 neutralization reduced graft infiltration in all instances, consistent with previous reports of IL-6 promoting leukocyte migration (34, 55–57). Previous investigations have highlighted the specific importance of IL-6 trans-signaling (IL-6 and soluble IL-6 receptor) in lymphocyte recruitment by promoting the production of ICAM-1 by endothelial cells (36, 57). A feature of IL-6 neutralization is the ability to inhibit both cis- and trans–IL-6 signaling. We were unable to find differences in intragraft ICAM-1 transcript expression between anti–IL-6 and rat IgG-treated controls (data not shown). Thus, IL-6 induction of MCP-1 may be more important in driving lymphocyte accumulation in the setting of transplant infiltration.

A second consistent feature of IL-6 neutralization was reduced levels of Th1 priming in recipient splenocytes in both models of acute rejection, suggesting that IL-6 promotes Th1 responses in vivo. These observations are consistent with previous in vivo observations of IL-6 promoting Th1 responses associated with
immune responses in experimental models of tuberculosis or leishmaniasis (58–61) and Th1 cell-mediated colitis (10). However, these findings are inconsistent with previous in vitro (5–7) and in vivo studies (62) implicating IL-6 as a promoter of Th2 differentiation. Such differences might be attributable to unique immune responses examined in contrasting settings, supporting the notion that the role of IL-6 in Th cell differentiation is contextually dependent. Although the causative effects of Th1/Th2 dominance in transplantation have been questioned (25, 63, 64), these findings correlate a shift toward less Th1 priming relative to Th2 with increased graft survival. Note that some graft-reactive Th2 clones reportedly have a regulatory effect (65). Recent evidence also indicates that Th2-associated cytokines, including IL-4, have protective effects on vascular endothelial cells by decreasing their sensitivity to complement-mediated killing (66, 67). Thus, skewing anti-graft responses toward Th2 cytokines may be graft protective, but as Th2 responses adeptly destroy grafts (24, 26, 31, 37) this protective effect is likely context-dependent.

In summary, our in vivo observations demonstrate the importance of IL-6 in mediating graft rejection by both CD8+ and CD4+ cells and that these two cell types have differential requirements for IL-6 in the process. Neutralization of IL-6 reduced chemokine expression, graft infiltration, and alloantibody production while skewing Th development from Th1 toward Th2. IL-6 has received much attention as a deciding factor in the reciprocal Th17 and regulatory T cell lineages (8) and as a suppressor of regulatory T cell function (68–70); however, our findings highlight a function of IL-6 in promoting in vivo Th1 immune responses. Collectively, these and our previous findings (18, 19) suggest that IL-6 signaling pathways may be a promising target for augmenting curative pathways in the development of interleukin 17-producing T helper cells. IL-6 has received much attention as a deciding factor in the reciprocal Th17 and regulatory T cell lineages and its role in Th17 cell development is well-established. 

Disclosures

The authors have no financial conflicts of interest.

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